


RESEARCH PAPER



7-Acylamino-3H-1,2-benzoxathiepine 2,2-dioxides as new isoform-selective carbonic anhydrase IX and XII inhibitors

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ABSTRACT

A series of 3H-1,2-benzoxathiepine 2,2-dioxides incorporating 7-acylamino moieties were obtained by an original procedure starting from 5-nitrosalicylaldehyde, which was treated with propenylsulfonyl chloride followed by Wittig reaction of the bis-olefin intermediate. The new derivatives, belonging to the homosulfocoumarin chemotype, were assayed as inhibitors of the zinc metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1). Four pharmacologically relevant human (h) isoforms were investigated, the cytosolic hCA I and II and the transmembrane, tumour-associated hCA IX and XII. No relevant inhibition of hCA I and II was observed, whereas some of the new derivatives were effective, low nanomolar hCA IX/XII inhibitors, making them of interest for investigations in situations in which the activity of these isoforms is overexpressed, such as hypoxic tumours, arthritis or cerebral ischaemia.

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KEYWORDS

Carbonic anhydrase; transmembrane isoforms; sulfocoumarin; homosulfocoumarin; isoform-selective inhibitor

1. Introduction

Sulfocoumarins (1,2-benzoxathiine 2,2-dioxides) and homosulfocoumarins (3H-1,2-benzoxathiepine 2,2-dioxides)^{1–5} are among the most investigated new classes of carbonic anhydrase (CA, EC 4.2.1.1) inhibitors, which have been designed considering the structurally similar coumarins^{6–8} as lead molecules. Indeed, CAs are widely spread enzymes in organisms of all types, from simple to complex ones^{9–15}, and are involved in crucial physiological processes, among which carbon fixation in diatoms and other marine organisms in which several genetic families of such metalloenzymes were reported⁹. In protozoans, CAs are involved in biosynthetic reactions⁹ whereas in bacteria, where at least three genetic families were described (α -, β -, and γ -CAs) these enzymes play crucial roles related both to metabolism but also virulence and survival in various niches¹⁰. In vertebrates, including humans, a high number of different CA isoforms belonging to the α -CA class were described^{11,12}, which by hydrating CO₂ to a weak base (bicarbonate) and a strong acid (hydronium ions), are involved in a multitude of processes, starting with pH regulation and ending with metabolism^{13,14}. As thus, CAs are drug targets for decades, with their inhibitors having pharmacological applications in a multitude of fields^{11–16}. The primary sulphonamides were discovered as CA inhibitors (CAIs) in the '40s, and most of the drugs that were launched in the next decades as diuretics, antiepileptics, or anti-glaucoma agents belonged to this class of compounds or to their isosteres such as the sulfamates and sulfamides¹¹. An important drawback of such first generation CA inhibitors (CAIs) was their lack of isoform selectivity, considering the fact that in humans at

least 12 catalytically active and three acatalytic isoforms are present^{11,12}. However, the new generation CAIs to which coumarins and sulfocoumarins belong, show significant isoform-selective inhibition profiles, as demonstrated in a considerable number of studies^{1–8}. This is principally due to the fact that these compounds possess a distinct inhibition mechanism compared to the sulphonamides, which coordinate to the zinc ion from the CA active site as anions^{11,12}. In fact, coumarins and sulfocoumarins act as prodrug inhibitors, undergoing an active site mediated hydrolysis, which leads to the formation of 2-hydroxy-cinnamic acids in the case of the coumarins, and ethane-sulphonates in the case of the sulfocoumarins, which subsequently bind in different active site regions, different of those where the classical sulphonamide CAIs bind^{1–8}. As shown by X-ray crystallography, the hydrolysed coumarins occlude the entrance of the CA active site cavity⁶, whereas the sulfocoumarins bind deeper within the active site, but still do not coordinate to the metal ion. Instead, the formed sulphonates anchor to the zinc-coordinated water molecule, as shown again by means of X-ray crystallographic techniques². As these regions of the CA active site are the most variable ones, a straightforward explanation of the isoform selectivity of these new generation CAIs was furnished by using a combination of crystallographic and kinetic studies, which also allowed the development of compounds showing a higher degree of selectivity^{15,16}. This allowed for the development of inhibitors useful for new pharmacological applications such as antitumor/antimetastatic compounds¹³, CAIs useful for the management of arthritis¹⁷, neuropathic pain¹⁸, and cerebral ischaemia¹⁹.

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Considering our interest in designing non-sulphonamide CAs with various potential applications, we report here a new series of homosulfocoumarins and their inhibitory profiles against the major human (h) CA isoforms, hCA I, II, IX, and XII, involved in many pathologies, including cancer.

2. Experimental part

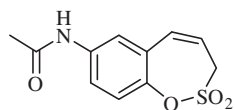
2.1. Chemistry

Reagents, starting materials/intermediates **1–7** and solvents were obtained from commercial sources (Sigma-Aldrich, St. Louis, MO) and used as received. Anhydrous CH_2Cl_2 and toluene were obtained by passing commercially available solvents through activated alumina columns. Thin-layer chromatography was performed on silica gel, spots were visualised with UV light (254 and 365 nm). Melting points were determined on an OptiMelt automated melting point system. IR spectra were recorded on Shimadzu FTIR IR Prestige-21 spectrometer. NMR spectra were recorded on Bruker Avance Neo (400 MHz) spectrometer with chemical shifts values (δ) in ppm relative to TMS using the residual DMSO-d_6 signal (^1H 2.50; ^{13}C 39.52) or CDCl_3 signal (^1H 7.26; ^{13}C 77.16) as an internal standard. High-resolution mass spectra (HRMS) were recorded on a mass spectrometer with a Q-TOF micro mass analyser using the ESI technique.

General procedure for synthesis of acyl compound **8–17**

To a solution of amino derivative **7** (1.0 eq.) in dry CH_2Cl_2 (20 ml per mmol of compound **7**) at 0°C appropriate acyl chloride (1.1 eq.) and NEt_3 (1.1 eq.) were added. The resulting mixture was stirred at room temperature under an argon atmosphere for 2 h. Water was added (20 ml per mmol of compound **7**). Layers were separated, water layer was washed with EtOAc (2×40 ml). Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , filtered, evaporated. The crude solids were recrystallised from EtOAc/petrol ether mixture to afford product.

N-(2,2-Dioxido-3H-1,2-benzoxathiepin-7-yl)acetamide (**8**).



Compound **8a** was prepared according to the general procedure from amino derivative **7** (150 mg; 0.71 mmol), acetyl chloride (56 μL ; 0.78 mmol) and Et_3N (110 μL ; 0.78 mmol) as white solid (127 mg; 70%). Mp $164\text{--}165^\circ\text{C}$.

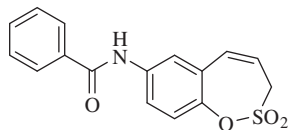
IR (film, cm^{-1}) $\nu_{\text{max}} = 3276$ (N–H), 1670 (C=O), 1370 (S=O), 1361 (S=O), 1166 (S=O), 1162 (S=O);

^1H NMR (400 MHz, DMSO-d_6) $\delta = 2.06$ (s, 3H), 4.37–4.41 (m, 2H), 5.96–5.604 (m, 1H), 6.89 (d, 1H, $J = 11.3$ Hz), 7.28 (d, 1H, $J = 8.9$ Hz), 7.58 (dd, 1H, $J = 8.9, 2.5$ Hz), 7.69 (d, 1H, $J = 2.5$ Hz), 10.16 (s, 1H) ppm.

^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 24.0, 51.0, 120.6, 120.8, 122.7, 128.4, 131.5, 138.0, 142.2, 168.6$ ppm.

HRMS (ESI) $[\text{M} + \text{H}]^+$: m/z calcd for $(\text{C}_{11}\text{H}_{12}\text{NO}_4\text{S})$ 254.0487. Found 254.0498.

N-(2,2-Dioxido-3H-1,2-benzoxathiepin-7-yl)benzamide (**9**).



Compound **9** was prepared according to the general procedure from amino derivative **7** (150 mg; 0.71 mmol), benzoyl chloride (90 μL ; 0.78 mmol) and

Et_3N (110 μL ; 0.78 mmol) as white solid (162 mg; 72%). Mp $174\text{--}175^\circ\text{C}$.

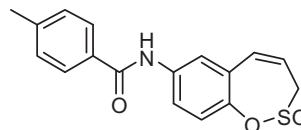
IR (film, cm^{-1}) $\nu_{\text{max}} = 3289$ (N–H), 1652 (C=O), 1370 (S=O), 1363 (S=O), 1163 (S=O);

^1H NMR (400 MHz, DMSO-d_6) $\delta = 4.43$ (dd, 2H, $J = 6.0, 0.9$ Hz), 5.99–6.06 (m, 1H), 6.93 (d, 1H, $J = 11.2$ Hz), 7.35 (d, 1H, $J = 8.8$ Hz), 7.52–7.58 (m, 2H), 7.59–7.64 (m, 1H), 7.82 (dd, 1H, $J = 8.8, 2.5$ Hz), 7.91 (d, 1H, $J = 2.5$ Hz), 7.94–7.99 (m, 2H), 10.46 (s, 1H) ppm.

^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 51.1, 120.9, 122.0, 122.1, 122.6, 127.7, 128.3, 128.5, 131.4, 131.8, 134.6, 137.9, 142.7, 165.7$ ppm

HRMS (ESI) $[\text{M} + \text{H}]^+$: m/z calcd for $(\text{C}_{16}\text{H}_{14}\text{NO}_4\text{S})$ 316.0644. Found 316.0654.

N-(2,2-Dioxido-3H-1,2-benzoxathiepin-7-yl)-4-methylbenzamide (**10**).



Compound **10** was prepared according to the general procedure from amino derivative **7** (150 mg; 0.71 mmol), 4-methylbenzoyl chloride (103 μL ; 0.78 mmol) and Et_3N (110 μL ; 0.78 mmol) as white crystals (170 mg; 73%). Mp $197\text{--}198^\circ\text{C}$.

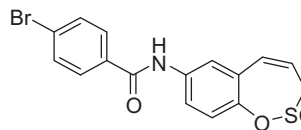
IR (film, cm^{-1}) $\nu_{\text{max}} = 3324$ (N–H), 1646 (C=O), 1378 (S=O), 1363 (S=O), 1177 (S=O), 1169 (S=O);

^1H NMR (400 MHz, DMSO-d_6) $\delta = 2.39$ (s, 3H), 4.41–4.45 (m, 2H), 5.99–6.06 (m, 1H), 6.92 (d, 1H, $J = 11.2$ Hz), 7.32–7.37 (m, 3H), 7.82 (dd, 1H, $J = 8.9, 2.6$ Hz), 7.86–7.92 (m, 3H), 10.37 (s, 1H) ppm

^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 21.0, 51.1, 120.8, 121.9, 122.1, 122.6, 127.7, 128.3, 129.0, 131.4, 131.6, 138.0, 141.9, 142.6, 165.5$ ppm

HRMS (ESI) $[\text{M} + \text{H}]^+$: m/z calcd for $(\text{C}_{17}\text{H}_{16}\text{NO}_4\text{S})$ 330.0800. Found 330.0815.

N-(2,2-Dioxido-3H-1,2-benzoxathiepin-7-yl)-4-bromobenzamide (**11**).



Compound **11** was prepared according to the general procedure from amino derivative **7** (150 mg; 0.71 mmol), 4-bromobenzoyl chloride (171 mg; 0.78 mmol) and Et_3N (110 μL ; 0.78 mmol) as white solid (166 mg; 59%). Mp $185\text{--}186^\circ\text{C}$.

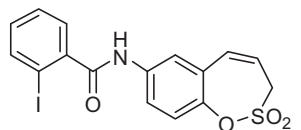
IR (film, cm^{-1}) $\nu_{\text{max}} = 3260$ (N–H), 1653 (C=O), 1375 (S=O), 1363 (S=O), 1167 (S=O);

^1H NMR (400 MHz, DMSO-d_6) $\delta = 4.42\text{--}4.46$ (m, 2H), 5.99–6.06 (m, 1H), 6.92 (d, 1H, $J = 11.3$ Hz), 7.35 (d, 1H, $J = 8.8$ Hz), 7.74–7.83 (m, 3H), 7.88–7.94 (m, 3H), 10.52 (s, 1H) ppm

^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 51.2, 120.9, 122.0, 122.2, 122.7, 125.6, 128.3, 129.8, 131.4, 131.5, 133.6, 137.7, 142.8, 164.7$ ppm

HRMS (ESI) $[\text{M} + \text{H}]^+$: m/z calcd for $(\text{C}_{16}\text{H}_{13}\text{BrNO}_4\text{S})$ 393.9749. Found 393.9736.

N-(2,2-Dioxido-3H-1,2-benzoxathiepin-7-yl)-2-iodobenzamide (**12**).



Compound **12** was prepared according to the general procedure from amino derivative **7** (150 mg; 0.71 mmol), 2-iodobenzoyl chloride (208 mg; 0.78 mmol) and Et_3N (110 μL ; 0.78 mmol) as white solid (276 mg; 88%). Mp $188\text{--}189^\circ\text{C}$.

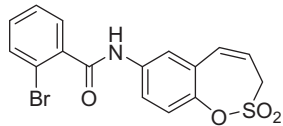
IR (film, cm^{-1}) $\nu_{\text{max}} = 3240$ (N–H), 1641 (C=O), 1374 (S=O), 1362 (S=O), 1156 (S=O);

^1H NMR (400 MHz, DMSO- d_6) δ = 4.41–4.45 (m, 2H), 6.00–6.08 (m, 1H), 6.94 (d, 1H, J = 11.2 Hz), 7.22–7.28 (m, 1H), 7.36 (d, 1H, J = 8.8 Hz), 7.47–7.55 (m, 2H), 7.72 (dd, 1H, J = 8.8, 2.5 Hz), 7.87 (d, 1H, J = 2.5 Hz), 7.9–7.97 (m, 1H), 10.67 (s, 1H) ppm

^{13}C NMR (100 MHz, DMSO- d_6) δ = 51.0, 93.6, 121.0, 121.2, 121.3, 122.9, 128.1, 128.2, 128.5, 131.2, 131.5, 137.7, 139.1, 142.7, 142.8, 167.7 ppm

HRMS (ESI) $[M + H]^+$: m/z calcd for ($\text{C}_{16}\text{H}_{13}\text{INO}_4\text{S}$) 441.9610 Found 441.9609.

N-(2,2-Dioxido-3H-1,2-benzoxathiepin-7-yl)-2-bromobenzamide (13).



Compound **13** was prepared according to the general procedure from amino derivative **7** (150 mg; 0.71 mmol), 2-bromobenzoyl chloride (102 μL ; 0.78 mmol) and Et_3N (110 μL ; 0.78 mmol) as white solid (230 mg; 82%). Mp 177–178 $^\circ\text{C}$.

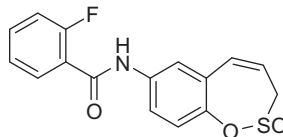
IR (film, cm^{-1}) ν_{max} = 3288 (N–H), 1653 (C=O), 1371 (S=O), 1176 (S=O), 1156 (S=O);

^1H NMR (400 MHz, DMSO- d_6) δ = 4.43 (dd, 2H, J = 6.0, 0.9 Hz), 6.00–6.07 (m, 1H), 6.94 (d, 1H, J = 11.2 Hz), 7.36 (d, 1H, J = 8.9 Hz), 7.41–7.47 (m, 1H), 7.51 (dt, 1H, J = 7.4, 1.1 Hz), 7.55–7.59 (m, 1H), 7.69–7.76 (m, 2H), 7.87 (d, 1H, J = 2.6 Hz), 10.73 (s, 1H) ppm

^{13}C NMR (100 MHz, DMSO- d_6) δ = 51.0, 118.9, 121.1, 121.2, 122.9, 127.8, 128.6, 128.9, 131.4, 131.5, 132.8, 137.6, 138.8, 142.8, 166.0 ppm

HRMS (ESI) $[M + H]^+$: m/z calcd for ($\text{C}_{16}\text{H}_{13}\text{BrNO}_4\text{S}$) 393.9749 Found 393.9766.

N-(2,2-Dioxido-3H-1,2-benzoxathiepin-7-yl)-2-fluorobenzamide (14).



Compound **14** was prepared according to the general procedure from amino derivative **7** (150 mg; 0.71 mmol), 2-fluorobenzoyl chloride (93 μL ; 0.78 mmol) and Et_3N (110 μL ; 0.78 mmol) as white solid (188 mg; 79%). Mp 173–174 $^\circ\text{C}$.

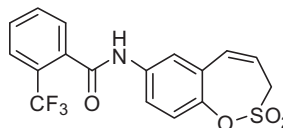
IR (film, cm^{-1}) ν_{max} = 1671 (C=O), 1371 (S=O), 1365 (S=O), 1164 (S=O) 1156 (S=O);

^1H NMR (400 MHz, DMSO- d_6) δ = 4.41–4.45 (m, 2H), 6.00–6.07 (m, 1H), 6.93 (d, 1H, J = 11.2 Hz), 7.32–7.40 (m, 3H), 7.56–7.63 (m, 1H), 7.65–7.71 (m, 1H), 7.74 (dd, 1H, J = 8.8, 2.5 Hz), 7.86 (d, 1H, J = 2.5 Hz), 10.65 (s, 1H) ppm

1. ^{13}C NMR (100 MHz, DMSO- d_6) δ = 51.1, 116.2 (d, J = 21.7 Hz), 121.0, 121.4, 121.5, 122.8, 124.6 (d, J = 5.5 Hz), 124.7 (d, J = 6.3 Hz), 128.5, 129.9 (d, J = 2.6 Hz), 131.4, 132.8 (d, J = 8.5 Hz), 137.5, 142.8, 159.9 (d, J = 249 Hz), 163.0 ppm

2. HRMS (ESI) $[M + H]^+$: m/z calcd for ($\text{C}_{16}\text{H}_{13}\text{FNO}_4\text{S}$) 334.0549 Found 334.0554.

N-(2,2-Dioxido-3H-1,2-benzoxathiepin-7-yl)-2-(trifluoromethyl)benzamide (15).



Compound **15** was prepared according to the general procedure from amino derivative **7** (150 mg; 0.71 mmol), 2-(trifluoromethyl)benzoyl chloride (115 μL ; 0.78 mmol)

and Et_3N (110 μL ; 0.78 mmol) as white solid (236 mg; 87%). Mp 192–193 $^\circ\text{C}$.

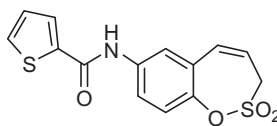
IR (film, cm^{-1}) ν_{max} = 3195 (N–H), 1666 (C=O), 1377 (S=O), 1316 (S=O), 1164 (S=O);

^1H NMR (400 MHz, DMSO- d_6) δ = 4.42–4.45 (m, 2H), 6.00–6.08 (m, 1H), 6.94 (d, 1H, J = 11.2 Hz), 7.36 (d, 1H, J = 8.8 Hz), 7.67–7.76 (m, 3H), 7.78–7.89 (m, 3H), 10.81 (s, 1H) ppm

^{13}C NMR (100 MHz, DMSO- d_6) δ = 51.0, 121.1, 121.3, 122.9, 123.8 (q, J = 274 Hz), 125.8 (q, J = 31.2 Hz), 126.4 (q, J = 4.6 Hz), 128.5, 128.6, 130.3, 131.4, 132.7, 135.8 (q, J = 2.3 Hz), 137.6, 142.8, 165.8 ppm

HRMS (ESI) $[M + H]^+$: m/z calcd for ($\text{C}_{17}\text{H}_{13}\text{NO}_4\text{F}_3\text{S}$) 384.0517 Found 384.0519.

N-(2,2-Dioxido-3H-1,2-benzoxathiepin-7-yl)thiophene-2-carboxamide (16).



Compound **16** was prepared according to the general procedure from amino derivative **7** (150 mg; 0.71 mmol), 2-thiophenecarbonyl chloride (84 μL ; 0.78 mmol) and Et_3N (110 μL ; 0.78 mmol) as white solid (185 mg; 81%). Mp 162–163 $^\circ\text{C}$.

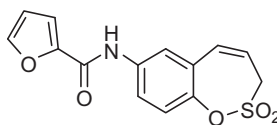
IR (film, cm^{-1}) ν_{max} = 3357 (N–H), 1648 (C=O), 1372 (S=O), 1356 (S=O), 1178 (S=O), 1165 (S=O);

^1H NMR (400 MHz, DMSO- d_6) δ = 4.44 (dd, 2H, J = 6.0, 1.1 Hz), 5.99–6.06 (m, 1H), 6.92 (d, 1H, J = 11.2 Hz), 7.23–7.26 (m, 1H), 7.35 (d, 1H, J = 8.8 Hz), 7.78 (dd, 1H, J = 8.8, 2.6 Hz), 7.84 (d, 1H, J = 2.6 Hz), 7.88 (dd, 1H, J = 5.0, 1.1 Hz), 8.04 (dd, 1H, J = 3.8, 1.1 Hz), 10.43 (s, 1H) ppm

^{13}C NMR (100 MHz, DMSO- d_6) δ = 51.2, 120.9, 121.9, 122.1, 122.7, 128.2, 128.4, 129.5, 131.3, 132.3, 137.5, 139.5, 142.7, 160.0 ppm

HRMS (ESI) $[M + H]^+$: m/z calcd for ($\text{C}_{14}\text{H}_{12}\text{NO}_4\text{S}_2$) 322.0208 Found 322.0221.

N-(2,2-Dioxido-3H-1,2-benzoxathiepin-7-yl)furan-2-carboxamide (17).



Compound **17** was prepared according to the general procedure from amino derivative **7** (150 mg; 0.71 mmol), 2-furoyl chloride (84 μL ; 0.78 mmol) and Et_3N (110 μL ; 0.78 mmol) as white solid (185 mg; 81%). Mp 162–163 $^\circ\text{C}$.

IR (film, cm^{-1}) ν_{max} = 3299 (N–H), 1663 (C=O), 1367 (S=O), 1363 (S=O), 1165 (S=O), 1158 (S=O);

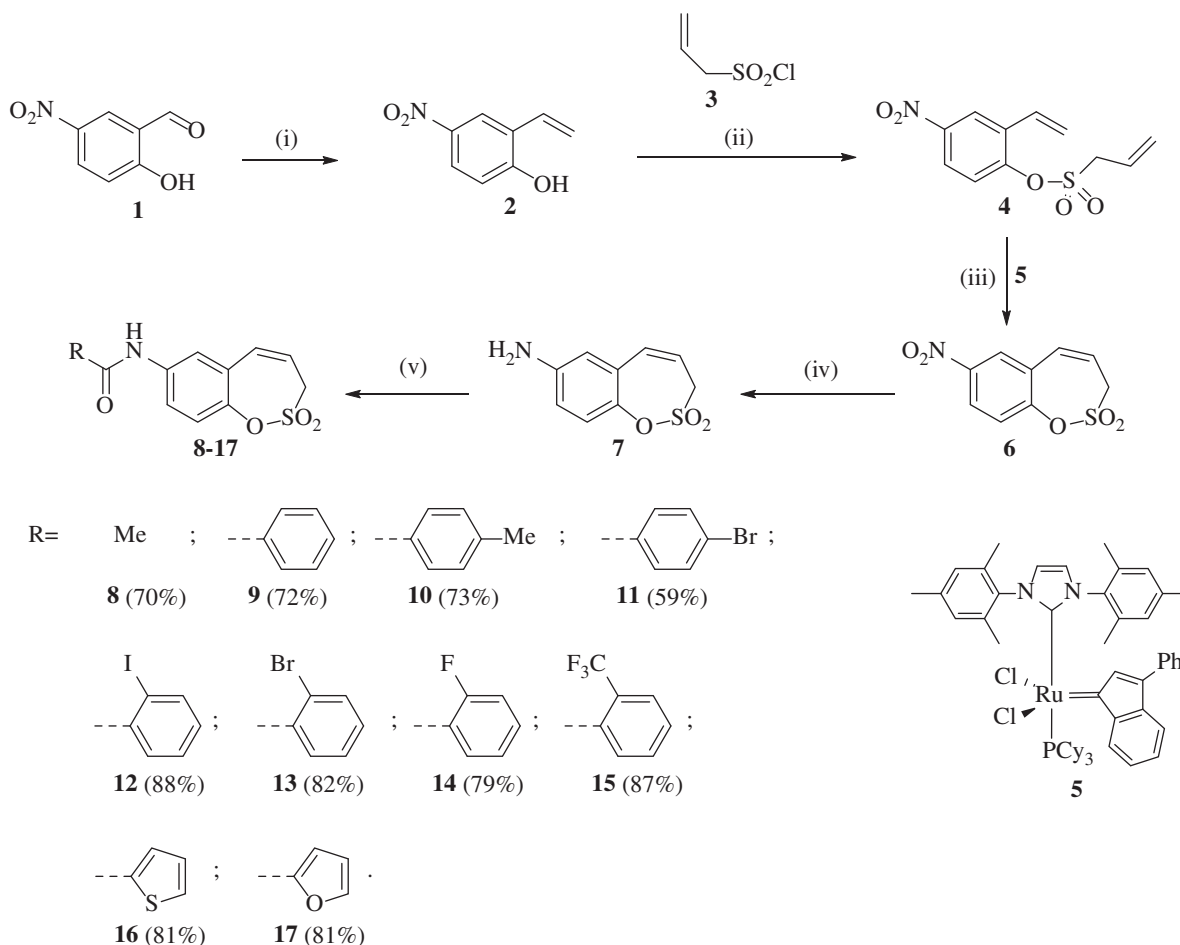
^1H NMR (400 MHz, DMSO- d_6) δ = 4.41–4.45 (m, 2H), 5.98–6.06 (m, 1H), 6.70–6.74 (m, 1H), 6.90 (d, 1H, J = 11.2 Hz), 7.32–7.38 (m, 2H), 7.80 (dd, 1H, J = 8.8, 2.6 Hz), 7.87 (d, 1H, J = 2.6 Hz), 7.95–7.97 (m, 1H), 10.41 (s, 1H) ppm

^{13}C NMR (100 MHz, DMSO- d_6) δ = 51.2, 112.3, 115.2, 120.9, 122.0, 122.2, 122.6, 128.3, 131.4, 137.3, 142.7, 146.0, 147.2, 156.3 ppm

HRMS (ESI) $[M + H]^+$: m/z calcd for ($\text{C}_{14}\text{H}_{12}\text{NO}_5\text{S}$) 306.0436 Found 306.0463.

2.2. CA inhibitory assay

An applied photophysics stopped-flow instrument has been used for assaying the CA catalysed CO_2 hydration activity²⁰. Phenol red (at a concentration of 0.2 mM) was used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalysed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor, at least six traces of the initial 5–10% of the reaction have been used for



Scheme 1. Reagents and conditions: (i) MePPh_3Br , tBuOK , THF, RT, 18 h, 65%; (ii) Net_3 , CH_2Cl_2 , 0°C to RT, 4 h, 57%; (iii) **5**, toluene, 70°C , 4 h, 96%; (iv) Fe, AcOH, EtOH, H_2O , 75°C , 1 h, 98%; (v) RCOCl , Net_3 , CH_2Cl_2 , 0°C to RT, 4 h.

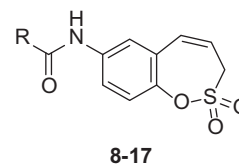
determining the initial velocity. The uncatalysed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled–deionised water, and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 6 h at room temperature prior to assay in order to allow for the formation of the E–I complex. The inhibition constants were obtained by nonlinear least-squares methods using PRISM 3 and the Cheng–Prusoff equation, as reported earlier^{21–23}, and represent the mean from at least three different determinations. All CA isoforms were recombinant ones obtained in-house as reported earlier^{21,24}.

3. Results and discussion

3.1. Chemistry

Starting from the benzaldehyde derivative **1**, the synthesis of the key intermediate **7** was reported earlier by our groups¹. Briefly, the synthesis of 7-amino-3H-1,2-benzoxathiepine 2,2-dioxide (**7**) was started with a Wittig reaction in which 5-nitro-salicylic aldehyde **1** was converted to the corresponding mono-olefin **2** in 65% yield (Scheme 1). Treatment of compound **2** with allyl sulphonyl chloride (**3**) provided the bisolefin **4** in 65% yield. In the next step, the olefin metathesis reaction with Ru-catalyst **5** was employed, leading to the conversion of compound **4** to 7-nitro-3H-1,2-benzoxathiepine 2,2-dioxide **6** in 96% yield. The nitro derivative **6** was thereafter reduced with iron in acidic medium to

Table 1. Inhibition data of human CA isoforms CA I, II, IX and XII with 3H-1,2-benzoxathiepine 2,2-dioxides **8–17** using acetazolamide (AAZ) as a standard drug.



Cmpd	R	K_i (nM) ^{a,b}			
		hCA I	hCA II	hCA IX	hCA XII
8	CH ₃	>100 μM	>100 μM	61.8	162.5
9	C ₆ H ₅	>100 μM	>100 μM	208.6	370.1
10	4-CH ₃ -C ₆ H ₄	>100 μM	>100 μM	83.0	309.3
11	4-Br-C ₆ H ₄	>100 μM	>100 μM	353.3	140.7
12	2-I-C ₆ H ₄	>100 μM	>100 μM	45.4	643.7
13	2-Br-C ₆ H ₄	>100 μM	>100 μM	66.8	96.2
14	2-F-C ₆ H ₄	>100 μM	>100 μM	74.6	40.3
15	2-CF ₃ -C ₆ H ₄	>100 μM	>100 μM	19.7	8.7
16	thien-2-yl	>100 μM	>100 μM	177.5	73.2
17	furan-2-yl	>100 μM	>100 μM	210.1	134.4
AAZ	–	250	12	25	5.7

^aMean from three different assays, by a stopped flow technique (errors were in the range of ± 5 –10% of the reported values).

^bIncubation time 6 h.

the corresponding amine **7** in nearly quantitative yield (98%). The key intermediate **7** was subsequently reacted with a series of acyl chlorides to afford the desired compounds **8–17** in good to

excellent yields (see Experimental for details). The nature of moieties R was chosen in such a way to assure chemical diversity. Apart R=Me in compound **8**, the remaining derivatives **9–17** incorporated aromatic or heterocyclic moieties, such as phenyl, 2- or 4-substituted phenyls, thienyl and furyl. We found out in previous papers^{1–3} that aryl or hetaryl moieties on the sulfocoumarin, homosulfocoumarin or coumarin ring⁶ systems lead to compounds with an effective inhibition profile against CA isoforms of pharmacologic interest, such as the tumour-associated ones CA IX and XII.

3.2. Carbonic anhydrase inhibition

The obtained homosulfocoumarins **8–17** were investigated for their CA inhibitory properties by using a stopped-flow CO₂ hydrase assay²⁰ and four human CA isoforms (hCA I, II, IX, and XII) known to be drug targets¹ (Table 1).

As seen from data of Table 1, derivatives **8–17** did not significantly inhibit the cytosolic isoforms hCA I and II, similar to other homosulfocoumarins, sulfocoumarins or coumarins investigated earlier^{1–8}. On the other hand, the transmembrane, tumour-associated isoforms hCA IX and XII were inhibited by all these compounds in the nanomolar range. For hCA IX the K_s were in the range of 19.7–353.3 nM whereas for hCA XII in the range of 8.7–643.7 nM (Table 1). The nature of the R moiety on the carboxamide functionality greatly influenced the inhibitory power. For hCA IX/XII the optimal substitution was that present in compound **15**, 2-trifluoromethylphenyl, whereas the one leading to the least effective inhibitor was the one with 4-bromophenylcarboxamide moiety (compound **9**) for hCA IX and 2-iodophenylcarboxamide (compound **12**) for hCA XII. Overall, all these new homosulfocoumarins act as isoform IX/XII selective CAIs over hCA I and II, which is highly desirable for these new chemotypes with enzyme inhibitory properties.

4. Conclusions

A series of 3H-1,2-benzoxathiepine 2,2-dioxides incorporating 7-acylamino moieties were obtained by an original procedure starting from 5-nitrosalicylaldehyde which was treated with propenyl-sulfonyl chloride followed by cyclisation through a Wittig reaction of the bis-olefin intermediate. The new derivatives, belonging to the homosulfocoumarin chemotype, were assayed as inhibitors of the zinc metalloenzyme CA. Four pharmacologically relevant human (h) isoforms were investigated, the cytosolic hCA I and II, and the transmembrane, tumour-associated hCA IX and XII. No relevant inhibition of hCA I and II was observed, whereas some of the new derivatives were effective, low nanomolar hCA IX/XII inhibitors, making them of interest for investigations in situations in which the activity of these isoforms is overexpressed, such as hypoxic tumours, arthritis or cerebral ischaemia.

Disclosure statement

The author(s) do not declare any conflict of interest.

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References

- (a) Pustenko A, Stepanovs D, Žalubovskis R, et al. 3H-1,2-benzoxathiepine 2,2-dioxides: a new class of isoform-selective carbonic anhydrase inhibitors. *J Enzyme Inhib Med Chem* 2017;32:767–75.; (b) Pustenko A, Nocentini A, Balašova A, et al. Aryl derivatives of 3H-1,2-benzoxathiepine 2,2-dioxide as carbonic anhydrase inhibitors. *J Enzyme Inhib Med Chem* 2020;35:245–54.
- Tars K, Vullo D, Kazaks A, et al. Sulfocoumarins (1,2-benzoxathiepine 2,2-dioxides): a class of potent and isoform-selective inhibitors of tumor-associated carbonic anhydrases. *J Med Chem* 2013;56:293–300.
- Tanc M, Carta F, Bozdog M, et al. 7-Substituted-sulfocoumarins are isoform-selective, potent carbonic anhydrase II inhibitors. *Bioorg Med Chem* 2013;21:4502–10.
- Nocentini A, Ceruso M, Carta F, Supuran CT. 7-Aryl-triazolyl-substituted sulfocoumarins are potent, selective inhibitors of the tumor-associated carbonic anhydrase IX and XII. *J Enzyme Inhib Med Chem* 2016;31:1226–33.
- Grandane A, Tanc M, Mannelli LDC, et al. Substituted sulfocoumarins are selective carbonic anhydrase IX and XII inhibitors with significant cytotoxicity against colorectal cancer cells. *J Med Chem* 2015;58:3975–83.
- (a) Maresca A, Temperini C, Vu H, et al. Non-zinc mediated inhibition of carbonic anhydrases: coumarins are a new class of suicide inhibitors. *J Am Chem Soc* 2009;131:3057–62; (b) Maresca A, Temperini C, Pochet L, et al. Deciphering the mechanism of carbonic anhydrase inhibition with coumarins and thiocoumarins. *J Med Chem* 2010;53:335–44; (c) Temperini C, Innocenti A, Scozzafava A, et al. The coumarin-binding site in carbonic anhydrase accommodates structurally diverse inhibitors: the antiepileptic lacosamide as an example. *J Med Chem* 2010;53:850–4; (d) Touisni N, Maresca A, McDonald PC, et al. Glycosylcoumarin carbonic anhydrase IX and XII inhibitors strongly attenuate the growth of primary breast tumors. *J Med Chem* 2011;54:8271–7.
- Zengin Kurt B, Sonmez F, Durdagi S, et al. Synthesis, biological activity and multiscale molecular modeling studies for coumaryl-carboxamide derivatives as selective carbonic anhydrase IX inhibitors. *J Enzyme Inhib Med Chem* 2017;32:1042–52.
- (a) Maresca A, Scozzafava A, Supuran CT. 7,8-disubstituted-but not 6,7-disubstituted coumarins selectively inhibit the transmembrane, tumor-associated carbonic anhydrase isoforms IX and XII over the cytosolic ones I and II in the low nanomolar/subnanomolar range. *Bioorg Med Chem Lett* 2010;20:7255–8; (b) Maresca A, Supuran CT. Coumarins incorporating hydroxy- and chloro-moieties selectively inhibit the transmembrane, tumor-associated carbonic anhydrase isoforms IX and XII over the cytosolic ones I and II. *Bioorg Med Chem Lett* 2010;20:4511–4.
- (a) Xu Y, Feng L, Jeffrey PD, et al. Structure and metal exchange in the cadmium carbonic anhydrase of marine

- diatoms. *Nature* 2008;452:56–61; (b) Del Prete S, Vullo D, Fisher GM, et al. Discovery of a new family of carbonic anhydrases in the malaria pathogen *Plasmodium falciparum*—the η -carbonic anhydrases. *Bioorg Med Chem Lett* 2014;24:4389–96; (c) Jensen EL, Clement R, Kosta A, et al. A new widespread subclass of carbonic anhydrase in marine phytoplankton. *ISME J* 2019;13:2094–106.
10. (a) Capasso C, Supuran CT. Anti-infective carbonic anhydrase inhibitors: a patent and literature review. *Expert Opin Ther Pat* 2013;23:693–704; (b) Capasso C, Supuran CT. An overview of the alpha-, beta- and gamma-carbonic anhydrases from Bacteria: can bacterial carbonic anhydrases shed new light on evolution of bacteria? *J Enzyme Inhib Med Chem* 2015;30:325–32; (c) Capasso C, Supuran CT. Bacterial, fungal and protozoan carbonic anhydrases as drug targets. *Expert Opin Ther Targets* 2015;19:1689–704; (d) Supuran CT, Capasso C. Biomedical applications of prokaryotic carbonic anhydrases. *Expert Opin Ther Pat* 2018;28:745–54; (e) Nishimori I, Minakuchi T, Morimoto K, et al. Carbonic anhydrase inhibitors: DNA cloning and inhibition studies of the alpha-carbonic anhydrase from *Helicobacter pylori*, a new target for developing sulfonamide and sulfamate gastric drugs. *J Med Chem* 2006;49:2117–26.
 11. (a) Supuran CT. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. *Nature Rev Drug Discov* 2008;7:168–81; (b) Alterio V, Di Fiore A, D'Ambrosio K, et al. Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms? *Chem Rev* 2012;112:4421–68; (c) Supuran CT. Structure and function of carbonic anhydrases. *Biochem J* 2016;473:2023–32; (d) Innocenti A, Gülçin I, Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors. Antioxidant polyphenols effectively inhibit mammalian isoforms I–XV. *Bioorg Med Chem Lett* 2010;20:5050–3.
 12. (a) Supuran CT. How many carbonic anhydrase inhibition mechanisms exist? *J Enzyme Inhib Med Chem* 2016;31:345–60; (b) Nocentini A, Supuran CT. Advances in the structural annotation of human carbonic anhydrases and impact on future drug discovery. *Expert Opin Drug Discov* 2019;14:1175–97; (c) Supuran CT. Advances in structure-based drug discovery of carbonic anhydrase inhibitors. *Expert Opin Drug Discov* 2017;12:61–88; (d) De Simone G, Supuran CT. (In)organic anions as carbonic anhydrase inhibitors. *J Inorg Biochem* 2012;111:117–29.
 13. (a) Supuran CT. Carbonic anhydrase inhibitors as emerging agents for the treatment and imaging of hypoxic tumors. *Expert Opin Investig Drugs* 2018;27:963–70; (b) Supuran CT. Carbonic anhydrase inhibitors and their potential in a range of therapeutic areas. *Expert Opin Ther Pat* 2018;28:709–12; (c) Supuran CT. Applications of carbonic anhydrases inhibitors in renal and central nervous system diseases. *Expert Opin Ther Pat* 2018;28:713–21; (d) Neri D, Supuran CT. Interfering with pH regulation in tumours as a therapeutic strategy. *Nature Rev Drug Discov* 2011;10:767–77; (e) Supuran CT, Alterio V, Di Fiore A, et al. Inhibition of carbonic anhydrase IX targets primary tumors, metastases, and cancer stem cells: three for the price of one. *Med Res Rev* 2018;38:1799–836.
 14. (a) Supuran CT. Carbonic anhydrases and metabolism. *Metabolites* 2018;8:25; (b) Supuran CT. Carbonic anhydrase inhibition and the management of hypoxic tumors. *Metabolites* 2017;7:E48; (c) Da'dara AA, Angeli A, Ferraroni M, et al. Crystal structure and chemical inhibition of essential schistosome host-interactive virulence factor carbonic anhydrase SmCA. *Commun Biol* 2019;2:333.
 15. (a) Supuran CT, Ilies MA, Scozzafava A. Carbonic anhydrase inhibitors. Part 29. Interaction of isozymes I, II and IV with benzamide-like derivatives. *Eur J Med Chem* 1998;33:739–52; (b) Köhler K, Hillebrecht A, Schulze Wischeler J, et al. Saccharin inhibits carbonic anhydrases: possible explanation for its unpleasant metallic aftertaste. *Angew Chem Int Ed Engl* 2007;46:7697–9; (c) Scozzafava A, Menabuoni L, Mincione F, et al. Carbonic anhydrase inhibitors: perfluoroalkyl/aryl-substituted derivatives of aromatic/heterocyclic sulfonamides as topical intraocular pressure-lowering agents with prolonged duration of action. *J Med Chem* 2000;43:4542–51; (d) Sentürk M, Gülçin I, Daştan A, et al. Carbonic anhydrase inhibitors. Inhibition of human erythrocyte isozymes I and II with a series of antioxidant phenols. *Bioorg Med Chem* 2009;17:3207–11.
 16. (a) Supuran CT, Altamimi ASA, Carta F. Carbonic anhydrase inhibition and the management of glaucoma: a literature and patent review 2013–2019. *Expert Opin Ther Pat* 2019;29:781–92; (b) Supuran CT. Carbon-versus sulphur-based zinc binding groups for carbonic anhydrase inhibitors? *J Enzyme Inhib Med Chem* 2018;33:485–95; (c) Bilginer S, Gul HI, Erdal FS, et al. Synthesis, cytotoxicities, and carbonic anhydrase inhibition potential of 6-(3-aryl-2-propenoyl)-2(3H)-benzoxazolones. *J Enzyme Inhib Med Chem* 2019;34:1722–9.
 17. (a) Margheri F, Ceruso M, Carta F, et al. Overexpression of the transmembrane carbonic anhydrase isoforms IX and XII in the inflamed synovium. *J Enzyme Inhib Med Chem* 2016;31:60–3; (b) Bua S, Di Cesare Mannelli L, Vullo D, et al. Design and synthesis of novel nonsteroidal anti-inflammatory drugs and carbonic anhydrase inhibitors hybrids (NSAIDs-CAIs) for the treatment of rheumatoid arthritis. *J Med Chem* 2017;60:1159–70; (c) Akgul O, Di Cesare Mannelli L, Vullo D, et al. Discovery of novel nonsteroidal anti-inflammatory drugs and carbonic anhydrase inhibitors hybrids (NSAIDs-CAIs) for the management of rheumatoid arthritis. *J Med Chem* 2018;61:4961–77.
 18. (a) Carta F, Di Cesare Mannelli L, Pinard M, et al. A class of sulfonamide carbonic anhydrase inhibitors with neuropathic pain modulating effects. *Bioorg Med Chem* 2015;23:1828–40; (b) Supuran CT. Carbonic anhydrase inhibition and the management of neuropathic pain. *Expert Rev Neurother* 2016;16:961–8.
 19. Di Cesare Mannelli L, Micheli L, Carta F, et al. Carbonic anhydrase inhibition for the management of cerebral ischemia: in vivo evaluation of sulfonamide and coumarin inhibitors. *Enzyme Inhib Med Chem* 2016;31:894–9.
 20. Khalifah RG. The carbon dioxide hydration activity of carbonic anhydrase. I. Stop-flow kinetic studies on the native human isoenzymes B and C. *J Biol Chem* 1971;246:2561–73.
 21. (a) Vermelho AB, da Silva Cardoso V, Ricci Junior E, et al. Nanoemulsions of sulfonamide carbonic anhydrase inhibitors strongly inhibit the growth of *Trypanosoma cruzi*. *J Enzyme Inhib Med Chem* 2018;33:139–46; (b) Nocentini A, Carta F, Tanc M, et al. Deciphering the mechanism of human carbonic anhydrases inhibition with sulfocoumarins: computational and experimental studies. *Chemistry* 2018;24:7840–4; (c) Awadallah FM, Bua S, Mahmoud WR, et al. Inhibition studies on a panel of human carbonic anhydrases with N1-substituted secondary sulfonamides incorporating thiazolinone or imidazolone-indole tails. *J Enzyme Inhib Med Chem* 2018;33:629–38.

22. (a) Bua S, Bozdog M, Del Prete S, et al. Mono- and di-thio-carbamate inhibition studies of the δ -carbonic anhydrase TveCA δ from the marine diatom *Thalassiosira weissflogii*. *J Enzyme Inhib Med Chem* 2018;33:707–13; (b) Ferraroni M, Gaspari R, Scozzafava A, et al. Dioxygen, an unexpected carbonic anhydrase ligand. *J Enzyme Inhib Med Chem* 2018;33:999–1005; (c) El-Gazzar MG, Nafie NH, Nocentini A, et al. Carbonic anhydrase inhibition with a series of novel benzenesulfonamide-triazole conjugates. *J Enzyme Inhib Med Chem* 2018;33:1565–74; (d) Akocak S, Lolak N, Bua S, Supuran CT. Discovery of novel 1,3-diaryltriazene sulfonamides as carbonic anhydrase I, II, VII, and IX inhibitors. *J Enzyme Inhib Med Chem* 2018;33:1575–80.
23. (a) Nocentini A, Bonardi A, Gratteri P, et al. Steroids interfere with human carbonic anhydrase activity by using alternative binding mechanisms. *J Enzyme Inhib Med Chem* 2018;33:1453–9; (b) Nocentini A, Trallori E, Singh S, et al. 4-Hydroxy-3-nitro-5-ureido-benzenesulfonamides selectively target the tumor-associated carbonic anhydrase isoforms IX and XII showing hypoxia-enhanced antiproliferative profiles. *J Med Chem* 2018;61:10860–74; (c) Chohan ZH, Munawar A, Supuran CT. Transition metal ion complexes of Schiff bases. Synthesis, characterization and antibacterial properties. *Met Based Drugs* 2001;8:137–43; (d) Oztürk Sarikaya SB, Topal F, Sentürk M, et al. In vitro inhibition of α -carbonic anhydrase isozymes by some phenolic compounds. *Bioorg Med Chem Lett* 2011;21:4259–62.
24. (a) Awadallah FM, Bua S, Mahmoud WR, et al. Inhibition studies on a panel of human carbonic anhydrases with N1-substituted secondary sulfonamides incorporating thiazolone or imidazolone-indole tails. *J Enzyme Inhib Med Chem* 2018;33:629–38; (b) Supuran CT, Clare BW. Carbonic anhydrase inhibitors. Part 57. Quantum chemical QSAR of a group of 1,3,4-thiadiazole and 1,3,4-thiadiazoline disulfonamides with carbonic anhydrase inhibitory properties. *Eur J Med Chem* 1999;34:41–50.